Remarks

The present application was filed as a divisional application to seek patent coverage for the subject matter of original claims 9-30, which were restricted out in the Office Action of June 16, 1993 in the parent application, Serial No. 08/034,460.

In the present preliminary amendment, the cross-reference to related applications has been modified to update and to complete it. Further, the specification has been amended to refer to the identification numbers of the corresponding sequences in the Sequence Listing filed concurrently herewith in response to the Patent and Trademark Office Notice of June 11, 1996. (A request for extension of time accompanies the Sequence Listing.) The claims have also been amended to define the invention more precisely with reference to a preferred embodiment. In particular, independent 9 has been modified to expressly recite the subject matter of claim 13, thus specifying that the analog of S1 is a polypeptide that differs in sequence from the naturally occurring S1 by the substitution of one or more amino acid residues in the region bounded by valine 7 and proline 14. This is in fact the region for which mutagenesis has been specifically exemplified by applicant. A similar change has been made in independent claim 19, which is directed to a vaccine composition containing the S1 analog. The terminology "enzymatic activity" in independent claim 19 has been changed to "enzymatic activities", to be consistent with the specification (see sentence bridging pages 6 and 7 therein; see also original claim 9 where this same phraseology is employed). Claim 19 has also been modified to require the presence of pertussis toxin subunits S2-S5 (this preferred embodiment was previously recited in claim 27).

In addition, claims 14, 24 and 30 have been amended to change their respective dependencies. Claims 12 and 22 have been modified to delete the word "substantially". Claim 17 has been reworded because the antecedent basis for the terminology "amino-terminus" as previously used in this claim was not clear. Claim 18 has been amended to insert a reference to the sequence number for the recited polypeptide. Claim 28 has been modified to be consistent with parent claim

19. In addition, claim 35 is being added to provide specific coverage for a vaccine composition containing the particular polypeptide of Figure 7 (this polypeptide also being the subject, by itself, of claim 18).

No new matter had been introduced by any of the changes in the originally filed claims or by the addition of the new claim.

In addition to the above mentioned changes, claims 13, 23 and 27 have been canceled to avoid redundancy of subject matter in view of the amendment of their parent claims. Also, claim 29 has been deleted as being immaterial to the preferred compositions now being prosecuted, and claims 31-34 have been canceled as relating to subject matter elected for prosecution in the parent application (individual subunits S2, S3, S4 and S5).

The patentability of the present claims (9-12, 14-22, 24-26, 28, 30 and 35) is discussed below. It is noted herein that these claims are directed to the polypeptide expression products of the DNA molecules elected for examination in the parent. Consequently, it is believed that some or all of the same issues regarding patentability may be regarded by the Examiner as also applicable herein. Accordingly, these issues will also addressed among the following remarks. As will be explained, a careful effort has been made to facilitate the prosecution by avoiding at least some of these issues through amendment of the claims.

I. Prosecution History in the Parent Application

Claims 1-8 and 31-34 (directed to DNA) were elected for examination in parent application Serial No. 08/034,460. In the Office Action of June 16, 1993, these claims were finally rejected under 35 U.S.C. §101 as reciting an invention that lacks a patentable utility, and under 35 U.S.C. §112, first paragraph, as being based on a specification that is non-enabling and does not describe the claimed invention adequately. With respect to the rejection based on 35 U.S.C. §101, the Examiner stated that "no evidence is presented to suggest that any recombinantly produced encoded protein elicits toxin-neutralizing antibodies in a treated

animal" and that the claimed recombinant DNA molecules which encode such proteins thus "have no patentable utility". (It is noted herein that this rejection was later withdrawn by the examiner upon appeal). With respect to the rejection based on 35 U.S.C. §112, first paragraph, the Examiner contended that there was no evidence the S1 analogs alone produce protective levels of antibody when injected into an animal. The Examiner regarded this as significant because the claims then at issue did not always require the presence of the other subunits. The Examiner also asserted that the specification did not provide adequate guidance to make other mutations in the recited valine 7 to proline 14 region, applicant having made "only a few", and that none had been tested *in vivo* to confirm the recited antibody-eliciting activity. (These restatements are based on the Examiner's Answer dated October 12, 1994, of record in the parent application)

In the same Office Action, claims 1-8 and 31-34 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite in the use of the language "at least a portion" and "or a fragment or derivative of said portion" (utilized in reference to the recited DNA molecules), and claim 4 was rejected as being indefinite in the use of the phrase "substantially inactive enzymatically".

In addition, Claims 1-8 separately were rejected under 35 U.S.C. §103 as being unpatentable over Nicosia et al I, Infection and Immunity, Volume 55, Number 4, pages 963-967 (1987) or Nicosia et al. II, Proceedings of the National Academy USA, Volume 83, pages 4631-4635 (1986), taken together with Zucker et al., Molecular Immunology, Volume 21, Number 9, pages 785-793 (1984) and Shortle, Annual Review of Genetics, Volume 15, pages 265-294 (1981). The Nicosia et al. references were relied on by the Examiner for their teachings regarding the gene cloning, expression and immunological properties of pertussis toxin. Zucker et al. was relied on as teaching methods for the identification of antigenic determinants applicable to pertussis toxin. Finally, Shortle was relied on for its disclosure of applicable mutagenesis techniques.

Also in the June 16, 1993, Office Action in the parent case, Claims 31-34 were rejected under 35 U.S.C. §103 as being unpatentable over Nicosia II when

read in view of Chen et al., Journal of Bacteriology, Volume 161, Number 2, pages 758-763 (1985). With respect to this rejection, the Examiner stated that Nicosia et al. teaches the nucleotide sequence of the pertussis toxin operon and that Chen et al's disclosure would have made it obvious to subclone each of the genes for subunits S2-S5.

Both of the above mentioned rejections based on 35 U.S.C. §103 were later withdrawn by the Examiner upon appeal.

II. The Claimed Invention

The present claims are directed to detoxified derivatives of pertussis exotoxin that are utilizable as an antigenic agent (alone or with other agents) in a vaccine for immunization against pertussis (Whooping cough disease). In the modified exotoxin of this invention, subunit S1 has been altered to create an analog by substituting for one or more amino acid residues in the valine-7 to proline-14 region of the native sequence, thereby abolishing the enzymatic activity associated with pertussis toxin reactogenicity. The resulting deactivated analogs, used in combination with the other subunits of pertussis toxin (i.e., S2-S5), are effective in eliciting an immunogenic response, while being potentially safer in avoiding untoward side effects that sometimes occur upon vaccination with conventional pertussis vaccines. Notably, each of these claims requires the presence of all of the five subunits of pertussis toxin, so there is no issue raised regarding the efficacy of the analog S1 subunit when used alone. The vaccine claims (19-22, 24-26, 28 and 30) employ the term "comprising" and are thus open to the inclusion of additional antigenic components normally employed in acellular pertussis vaccines to enhance the efficacy.

It should also be noted that none of the present claims recites any of the particular language deemed objectionable by the Examiner in the parent. Thus, the phrases "at least a portion" and "or a fragment or derivative of said portion" are absent from each of the claims. Moreover, the term "substantially" has been deleted from claims 12 and 22, where it originally appeared.

Apart from the prior art, this leaves for consideration the sufficiency of the specification and allowability of the claims under 35 U.S.C. §112, first paragraph. Notwithstanding the position taken by the Examiner in the parent case with respect to the DNA claims, the scope of the present claims is reasonable and fully supported by the description in the specification. The experimental results reported in Tables 1 and 2 (pages 27 and 29, respectively, of the specification) show that single and double substitutions of amino acid residues in the valine-7 to proline-14 region of S1, in accordance with this invention, cause a loss of ADP-ribosyltransferase activity *in vitro*. These changes include arginine-9 to lysine (6A-3/4-1), tyrosine-8 to leucine + arginine-9 to glutamic acid (6A-3/8-1), arginine-9 to asparagine + serine-12 to glycine (6A-3/7-2), and aspartic acid-11 to proline + proline-14 to aspartic acid (6A-3/6-1). Considering the limited scope of the specified valine-7 to proline-14 region (eight amino acid residues), and the number of examples given ranging from one end of this region to the other, claims of the present breadth are very reasonable and, moreover, are necessary to secure adequate protection for the invention.

Moreover, the fact that these results were generated *in vitro* should not preclude the claims from being patented. First, in regard to the enzymatic activity, it is perfectly reasonable to conclude that if these analogs are inactive *in vitro*, they will also be inactive *in vivo*. Moreover, the analogs should remain enzymatically inactive even when used in combination with the other subunits of pertussis toxin (S2-S5) in the form of a detoxified holotoxin (or "holotoxoid"). Thus, the only recited biological property that might be in issue is whether the holotoxoid will be effective to elicit an immunogenic response *in vivo,* that is to say, can it truly immunize against the disease as intended? The answer to this question is emphatically "yes", and this conclusion is based not only on *in vitro* data, but also on *in vivo* evidence from laboratory tests performed on mice using the arg9→lys analog described by applicant in his specification, as well as on human clinical data using a similar analog.

The *in vivo* results from mice are described in the article entitled "Contribution of the B Oligomer to the Protective Activity of Genetically Attenuated Pertussis Toxin", authored by Juan L. Arcinega et al. in Infection and Immunity, Volume 59, Number 10, pages 3407-3410 (1991), copy enclosed. The Arciniega et al. article reports that an arginine-9 to lysine analog of S1 (i.e. applicant's) was combined with native B oligomer (S2-S5) of pertussis toxin, and that the resulting mutant holotoxin completely protected mice against respiratory infection upon challenge with *Bordetella pertussis*.

Similar *in vivo* data appears in the patent literature with respect to other detoxified analogs of S1 tested in animals. In particular, the Examiner's attention is directed to United States Patent No. 5,085,862 (Klein et al.), where it is reported that mutant pertussis holotoxins in which the glutamic acid residue at position 129 of S1 has been replaced with glycine, glutamine or asparagine, induce the formation of neutralizing antibodies in mice (see Example XV in column 18 and Tables 3 and 4 in column 21).

Finally, the use of an acellular pertussis vaccine based on genetically detoxified pertussis toxin as a component has since been validated <u>in humans</u> as a viable alternative to "whole cell" vaccines and chemically- or thermally-inactivated acellular vaccines. See Rappuoli et al., International Archives of Allergy and Immunology, Volume 108, pages 327-333 (1995), and Rappuoli, Infectious Agents and Disease, Volume 5, pages 21-28 (1996), copies of which are enclosed. Notably, the Chiron-Biocene genetically detoxified pertussis vaccine that was successfully employed in these clinical trials uses an arg9→lys mutation (as disclosed in the first of the Rappuoli articles).

III. <u>The Prior Art</u>

The prior art references mentioned above were considered in the parent case with respect to DNA claims 1-8, with the Examiner having ultimately concluded that the claims were patentable over these references. The present claims are also believed by applicant to patentable over these references. At this

time, applicant wishes to call the present Examiner's attention to another reference, namely, Locht and Keith, Science, Volume 232, pages 1258-1264 (1986). This article was also cited in the Information Disclosure Statement filed with the present application.

The Locht/Keith reference discloses that subunit S1 of pertussis toxin contains two regions of eight amino acids each that are homologous to regions in the A subunit of cholera and *E. coli* heat labile toxins, these regions occurring at tyrosine-8 to proline-15 and valine-51 to arginine-58 (page 1261). It is further stated therein that "these regions may be part of functional domains responsible for the similar activity of all three toxins" (page 1261); and that by physical identification of the active sites for ADP-ribosylation in the S1 subunit or target cell binding subunits S2-S5 " it is now possible to modify those sites by site-directed mutagenesis of the *B. pertussis* genome" (page 1263). These teachings, while of interest, would not have made the present invention obvious. In particular, the authors fail to identify specific amino acid residues responsible for enzymatic activity, merely stating that the specified regions "may" be part of functional domains responsible for activity without offering any proof. Moreover, it would not have been obvious from these teachings that one could make discrete substitutions in applicant's prescribed valine-7 to proline-14 region to abolish the enzymatic activity, while maintaining the ability to elicit toxin-neutralizing antibodies, as required in the present claims.

IV. Closing Comments

The present application is part of a series of applications dating back to September 4, 1987, all of which have been diligently prosecuted. Applicant has had to endure the frustration of not seeing a single patent claim issue in the United States in all this time, despite repeated attempts to move the prosecution forward. During this same period, applicant has seen (1) the growing interest in the vaccine industry of genetic detoxification as a method for the development of safer, effective vaccines; (2) the issuance to other parties, here and in Europe, of numerous patents based essentially on the same principle first described by applicant in his September 4, 1987 patent application; see, for instance, European

Patent No. 0 322 115 (to Connaught Laboratories Ltd.) and European Patent No. 0 322 533 (to Sclavo) , as well as U.S. Patents 5,221,618; 5,244,657; 5,332,583; 5,358,868; and 5,433,945, in addition to the above mentioned Klein et al patent; (3) inquiries by several major vaccine companies concerning the availability for licensing of the present invention; and (4) the approval and commercialization in Italy by Chiron-Biocene (formerly, Sclavo) of a pertussis vaccine which utilizes applicant's arg9→lys mutation. In view of the comments presented herein, it would be entirely proper, and long overdue, for a patent to issue to applicant for this invention. An early and favorable action is requested.

Respectfully submitted,

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